stable line in screening compounds.

Summary. Of 3 tissue culture systems tested, namely, Rabbit Testicular, Primary Rabbit Kidney, and RK_{13}, the latter proved to be the most satisfactory for testing of inhibition of myxoma and fibroma viruses. Plaque formations by both viruses were completely or partially inhibited by compounds in the following order of effectiveness: CA, IUDR, AZUR, Methotrexate, BUDR, TA, NMITC, FU, FUDR. 6-MP and HBB exhibited moderate inhibition of myxoma virus, and weak variable inhibition of fibroma virus. The effective compounds were not virucidal and did not prevent adsorption of virus to cells.

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Erythropoiesis During Pregnancy and Lactation. I. Effect of Various Hormones on Erythropoiesis During Lactation.* (30572)

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Because lactating rats have been found to have an increased red cell volume(1), and lactating mouse plasma and prolactin stimulate erythropoiesis(2), the red cell volume (RCV) and plasma erythropoietic activity were measured in postpartum lactating and nonlactating mice. The effect on the RCV of various hormones, known to be active in maintenance of lactation, also was studied in postpartum nonlactating mice and the erythropoietic activities of these hormones were assayed in polycythemic mice.

Materials and methods. Inbred virgin Swiss mice with a gestation of 20 days were used for breeding purposes in these experiments. Parturition and day 0 are used interchangeably to designate 0-12 hours after birth. Groups of nonlactating mice were obtained by removing the newborn during this period. The RCV was determined in parturition mice and in lactating and nonlactating mice on the days shown in Fig. 1. All RCVs were calculated from the total blood volume (TBV), determined with the I^{131}-tagged albumin dilution technique(3) and from the hematocrit corrected for the total body/venous hematocrit ratio(4). The RCV values of normal and prolactin treated mice obtained by this method closely agreed with those previously obtained by use of the Cr-51 *in vitro* tagged erythrocyte technique(2) in normal and prolactin injected mice.

Plasma was collected and pooled separately from each group of mice shown in Fig. 2 and then assayed as previously described in polycythemic mice(2), using a 72-hour incorporation of Fe^{59} into erythrocytes as a measure of erythropoietic activity.

In a second group of experiments, non-lactating mice received subcutaneous injections of 250 or 500 μg of prolactin† for 15 days. Injection of 500 μg of prolactin was started at 0-12, 24-36 and 48-60 hours from parturition in order to determine if other factors present in the plasma of early postpartum mice enhance or inhibit the response to prolactin. Starting at 0-12 hours from birth, other groups of nonlactating mice were in-

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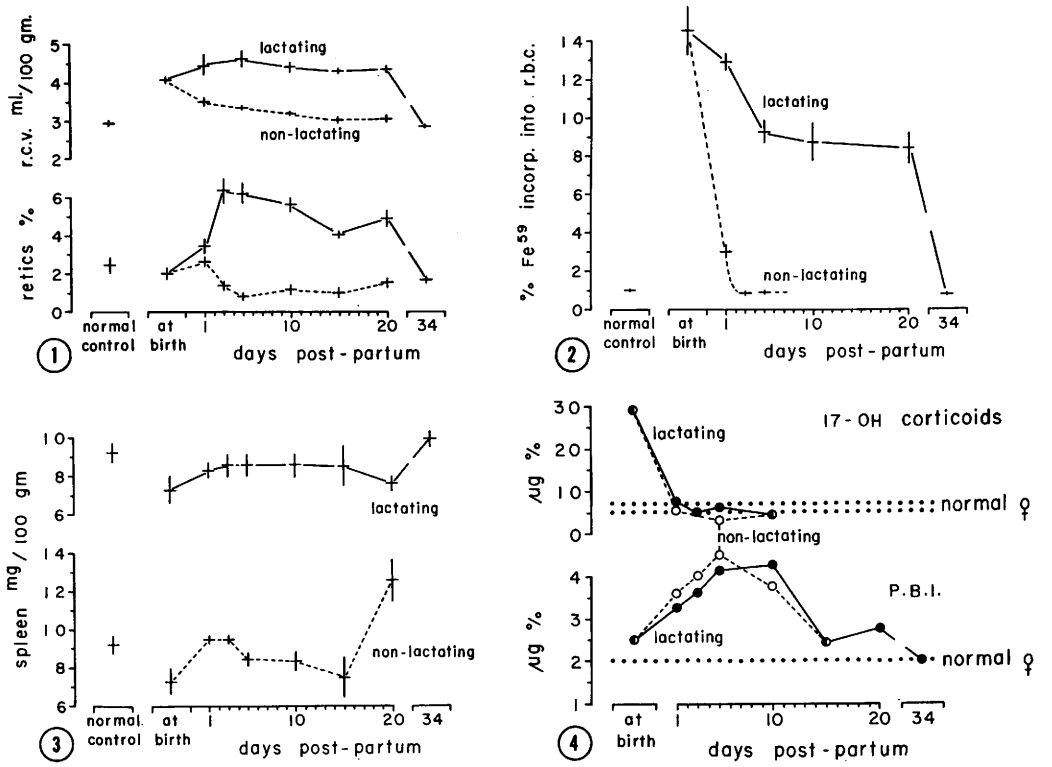

FIG. 1. Comparison of red cell volumes (RCV) and percent reticulocytes of lactating and nonlactating postpartum mice. Vertical bar represents mean ± standard error of each group (minimum of 5 mice/group). Lactating mice were weaned on 20th postpartum day. Normal control = nonpregnant normal female mice.

FIG. 2. 72-hr incorporation of Fe^{59} into RBC of polycythemic mice following injection of 1 ml of pooled plasma collected from each lactating and nonlactating group. Mice were injected on 4th and 5th posthypoxic days. Vertical bar represents standard error of mean of each group (minimum of 5 mice/group). Lactating mice were weaned on 20th postpartum day. Normal control = effect of plasma from normal nonpregnant female mice.

FIG. 3. Comparison of spleen weights (mg/100 g body wt) of lactating and nonlactating postpartum mice. Each group was composed of a minimum of 6, in most cases 20-25 splenic weights. Vertical bar represents mean ± standard error. Lactating mice were weaned on 20th postpartum day. Normal control = normal nonpregnant female mice.

FIG. 4. Comparison of plasma 17-OH corticoids and protein bound iodine (PBI) of lactating (●) and nonlactating (○)postpartum mice. Lactating mice were weaned on 20th postpartum day.

jected with 1-thyroxine and prednisolone acetate (Pred. Ac.) alone, in combination with each other, and in combination with prolactin in the doses listed in Table I.

Determinations of protein bound iodine (PBI)‡(5) and total plasma 17-OH corticoids‡(6) were done in duplicate on pooled plasma collected from the groups of lactating and nonlactating mice at various intervals postpartum.

‡ We wish to thank Dr. J. M. MacKenzie, Dr. S. Solomon and Dr. E. Venning for protein bound iodine and plasma 17-OH corticoids determinations.

Results. The RCV and reticulocytes of lactating mice increased significantly ($p < .001$) from parturition and achieved peak values by the fifth postpartum day (Fig. 1). During this period the plasma erythropoietic activity of these mice decreased moderately from its peak at parturition (Fig. 2). From the 5th to the 20th day of lactation the RCV and plasma erythropoietic activity maintained a constant and significant elevation as compared with the non-pregnant normal ($p < .001$) and there was a persistent reticulocytosis (Fig. 1). The lactating mice were

TABLE I. Comparison of RCV of Postpartum Nonlactating Mice Receiving Daily Injections of Hormones for 15 Days with Those of 15-Day Lactating and Nonlactating Mice Receiving No Injections.

Materials injected*	RCV, ml/100 g (± S.E.)	% Increase of RCV over those 15-day nonlactating mice receiving no injections	% Retics. (± S.E.)
15-day nonlactating†	3.01 ± .04 (8) ‡	—	1.00 ± .23
15-day lactating†	4.28 ± .05 (12)	42.2	4.02 ± .18
500 μg prolactin, hr from parturition			
(0-12)	4.17 ± .07 (10)	38.3	3.18 ± .22
(24-36)	4.39 ± .1 (8)	45.8	2.39 ± .27
(48-60)	4.19 ± .11 (7)	39.3	2.70 ± .41
250 μg prolactin	4.35 ± .11 (7)	44.5	2.30 ± .19
250 μg prolactin + 50 μg Pred. Ac. + 2.5 μg thyroxine	4.18 ± .07 (7)	38.9	2.17 ± .30
50 μg Pred. Ac.	3.08 ± .07 (9)	2.33	1.50 ± .32
5 μg (× 7d), 2.5 μg (× 8d), l-thyroxine	3.42 ± .08 (9)	13.62	1.13 ± .25
2-5 μg thyroxine + 50 μg Pred. Ac.	2.91 ± .03 (8)	.0	1.07 ± .12
250 μg prolactin + 2.5 μg thyroxine	3.51 ± .06 (7)	16.6	1.06 ± .27
250 μg prolactin + 50 μg Pred. Ac.	3.79 ± .09 (10)	25.9	1.40 ± .49

* Nonlactating mice weaned 0-12 hr from birth unless otherwise noted.
† Uninjected controls.
‡ In parentheses, No. of mice per group.

weaned on the 20th postpartum day. Fourteen days after weaning the RCV had returned to the nonlactating normal level, the reticulocytes had dropped to below the normal value and plasma erythropoietic activity had disappeared.

In nonlactating postpartum mice a rapid and significant decrease of the RCV ($p < .0025$) occurred 24 hours after removal of the newborn, followed by a more gradual decline through the 15th day to the nonpregnant normal level; this was associated with a drop of reticulocytes to below the level of the normal controls by the 5th day (Fig. 1). The plasma erythropoietic activity of these mice dropped rapidly within 24 hours after removal of the newborn and was absent by the 3rd postpartum day (Fig. 2). Both postpartum groups showed an increase of the splenic weights over those of the parturition mice (Fig. 3) which was only significant in the nonlactating mice ($p < .0025$) and paralleled their early postpartum decrease of RCV. The plasma 17-OH corticoids and PBI values of both groups were similar (Fig. 4).

The mean RCV of 15-day lactating mice was 42% greater than that of the 15-day nonlactating mice (Table I). Injection of prolactin daily for 15 days into nonlactating postpartum mice maintained essentially the same increase of RCV as was observed in the 15-day lactating mice and starting at 0-12, 24-36, or 48-60 hours from parturition caused no significant variation in the amount of increase (Table I). Daily injection of 250 μg of prolactin produced as great an increase of RCV as 500 μg daily. Similarly, triple injections daily of prolactin, l-thyroxine and Pred. Ac. maintained a RCV equivalent to that of the 15-day lactating mice (Table I), while injections of l-thyroxine and Pred. Ac., alone or in combination into nonlactating mice, resulted in either a slight or no increase of RCV. When prolactin was given in combination with either of these two hormones the erythropoietic stimulating effect was less than when prolactin was injected alone (Table I).

These hormones were assayed in polycythemic mice (Table II), both alone and in

the same combinations as above. Prolactin administration significantly increased the Fe^{59} incorporation ($p<.001$). Fifty micrograms of Pred. Ac. and 5 μg of 1-thyroxine, alone and in combination, had no effect. Increasing the dose of Pred. Ac. to 125 μg produced a significant response ($p<.001$). 1-Thyroxine appeared to inhibit the effect of prolactin while Pred. Ac. did not enhance or antagonize the erythropoietic effect of prolactin. The effect of injection of all 3 hormones was not significantly different from that of injection of prolactin alone.

TABLE II. 72-Hour Incorporation of Fe^{59} into Erythrocytes of Polycythemic Mice Injected with Hormones* (mean ± S.E.).

Group	Saline	250 μg prolactin	5 μg thyroxine	50 μg Pred. Ac.	125 μg Pred. Ac.	5 μg thyroxine + 50 μg Pred. Ac.	250 μg prolactin + 5 μg thyroxine	250 μg prolactin + 50 μg Pred. Ac.	250 μg prolactin + 50 μg Pred. Ac. + 5 μg thyroxine
% Fe^{59} RBC at 72 hr	.72 ± .13	1.96 ± .12	.53 ± .09	.43 ± .06	1.94 ± .12	.79 ± .05	.48 ± .04	1.90 ± .33	2.52 ± .44
	(3)†	(5)	(6)	(6)	(5)	(5)	(5)	(4)	(6)
P		<.001	>.05	>.05	<.001	>.05	>.05	>.05	<.001

* Injections on 4th and on 5th post-hypoxic day.
† No. of mice per group enclosed in parentheses.

Discussion. The increase of RCV and of reticulocytes during early lactation was associated with high plasma erythropoietic activity. The observed early moderate decline of this activity may have been due to increased utilization by the bone marrow secondary to increased erythropoiesis or to the disappearance of erythropoietic factor(s) carried over from late pregnancy. The latter consideration would seem to be relatively unimportant since 24-36 hours after parturition nonlactating plasma contained only 3% activity, hence a large portion of the increased erythropoietic activity of lactating plasma at this time must have come from the postpartum production of erythropoietic factor(s). The biological half-life of endogenous erythropoietin activity in the rat is 3-5 hours(7) and assuming that that of the mouse is similar and that lactation is necessary for the continued production of erythropoietic activity postpartum, any erythropoietin in the plasma carried over from late pregnancy should have disappeared in nonlactating mice by 24 hours; this is supported by the observed prompt cessation of erythropoiesis in these mice. Although the plasma 17-OH corticoids and PBI curves were similar in both lactating and nonlactating mice, the RCV and plasma erythropoietic activity of the nonlactating mice declined sharply, indicating that in the doses given these thyroid and adrenal hormones do not play a significant role in the production of lactational polycythemia. Furthermore, while injection of prolactin, which does not produce lactation *per se,* maintained the RCV of nonlactating mice, injection of Pred. Ac. and 1-thyroxine, alone or in combination, into nonlactating mice or into polycythemic mice did not effect the same change. Injection of all 3 hormones concurrently caused no greater increase of the RCV of postpartum mice or of the erythropoietic response of polycythemic mice than did injection of prolactin alone. Although 125 μg of Pred. Ac. produced a sig-

nificant erythropoietic response in polycythemic mice, this is an extremely high dose and normal female mice injected with 125 μg daily for 15 days had no increase of RCV (unpublished).

The erythropoietic effect of prolactin does not require the mediation of the testes(2). Estrogens(8), progesterone and growth hormone(2) have not been shown to stimulate erythropoiesis in the endocrinologically intact animal. Of the hormones not investigated, erythropoietin (ESF) cannot be excluded as a possible cause of the observed erythropoietic activity in lactating mice. Since the kidney is the major site of ESF production and prolactin has been shown to increase the glomerular filtration rate and renal plasma flow(9), the interrelationship of prolactin and ESF production is under investigation.

The decrease of RCV in nonlactating mice was more rapid and greater than could be explained by cessation of erythropoiesis alone. The associated increase of the splenic weights of these mice suggests that sequestration of erythrocytes in the spleen and their subsequent destruction early postpartum, may have been responsible in part or in whole for this rapid decrease of RCV.

Summary. RCV and plasma erythropoietic activity were significantly increased throughout lactation in the mouse as compared with normal female mice and with nonlactating postpartum mice. The RCV decreased and plasma erythropoietic activity disappeared rapidly in nonlactating mice. Prolactin stimulated erythropoiesis in postpartum nonlactating mice and polycythemic mice while adrenal steroids and thyroid hormone were ineffective.

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Effect of Sublethal Concentrations of Penicillins on the Lysis of Bacteria by Lysozyme and Trypsin. (30573)

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Previous studies from this laboratory(1-3) demonstrated that when strains of *Staphylococcus aureus* were grown in the presence of sublethal concentrations of nafcillin, the normally lysozyme-resistant *S. aureus* was rendered susceptible to partial lysis by lysozyme. Subsequently it was found that the organisms were almost completely lysed if trypsin and lysozyme were added successively to the cell suspensions(2). The precise relationship between the concentrations of nafcillin required to influence lytic response and to prevent growth has not been examined in detail; however, the results obtained with a number of semisynthetic penicillins suggest significant quantitative differences between the ability of nafcillin and the isoxazole penicillins to render *S. aureus* susceptible to enzymic action. Our observation has prompted speculation that these antibiotics may involve somewhat different mechanisms and/or primary sites of action on cell wall synthesis and that sublethal levels which inhibit synthesis of the mucopeptide polymer may play a role in pathogenesis by rendering the susceptible bacteria vulnerable to enzymic lysis.